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(54) Title: SALTS OF PEPTIDES WITH CARBOXY-TERMINATED POLYESTERS

(57) Abstract

This invention relates to novel salts composed of a cation derived from a peptide containing at least one basic group and an anion derived from a carboxy-terminated polyester, processes for the manufacture of such salts, and the use of such salts in the manufacture of extended release pharmaceutical compositions. The salts of the invention possess a variety of properties which are useful in the formulation of extended release pharmaceutical compositions, whether the salts are in pure form or are in admixture with either an excess of the peptide in its free, unbound form or an excess of the free polyester.

TITLE: SALTS OF PEPTIDES WITH CARBOXY-TERMINATED POLYESTERS

This invention relates to novel salts composed of a cation derived from a peptide containing at least one basic group and an anion derived from a carboxy-terminated polyester, processes for the manufacture of such salts, and the use of such salts in the manufacture of extended release pharmaceutical compositions. The salts of the invention possess a variety of properties which are useful in the formulation of extended release pharmaceutical compositions, whether the salts are in pure form or are in admixture with either an excess of the peptide in its free, unbound form or an excess of the free polyester. Such salts are amphipathic, being comprised in part of a peptide, which is hydrophilic and lipophobic, and in part a polyester, which is hydrophobic and lipophilic.

The word "peptide" is used herein in a generic sense to include poly(amino acids) which are normally generally referred to as "peptides", "polypeptides" or "proteins"; and a "basic peptide" is a peptide which is basic in nature, arising from the presence of an excess of basic amino acids, for example arginine or lysine, or arising from the N-terminus of the peptide, or simply a peptide which contains at least one basic group, optionally in the presence of one or more acidic amino acid groups. The term also includes synthetic analogues of peptides, unnatural amino acids having basic functionality, or any other form of introduced basicity. The word "polyester" is used hereinafter to mean a carboxy-terminated polyester.

European Patent No. 58,481 alludes to the possibility of specific chemical interactions between the terminal carboxylic acid group of a polyester and a basic group or groups within a peptide. Lawter et al., Proc. Int. Symp. Control Rel. Bioact. Mater., 14, 19, (1987) and Okada et al., Pharmaceutical Research, 8, 584-587 (1991), also refer to this possibility, but these publications are speculative in this regard, in

peptides which may be substantially stable in the extended release formulations over the intended period of release, and which may therefore be used in the compositions of this invention, are oxytocin, vasopressin, adrenocorticotrophic hormone (ACTH), epidermal growth factor (EGF), prolactin, luteinising hormone, follicle stimulating hormone, luliberin or luteinizing hormone releasing hormone (LHRH), insulin, somatostatin, glucagon, interferon, gastrin, tetragastrin, pentagastrin, urogastrone, secretin, calcitonin, enkephalins, endorphins, kyotorphin, taftsin, thymopoietin, thymosin, thymostimulin, thymic humoral factor, serum thymic factor, tumour necrosis factor, colony stimulating factors, motilin, bombesin, dinorphin, neurotensin, cerulein, bradykinin, urokinase, kallikrein, substance P analogues and antagonists, angiotensin II, nerve growth factor, blood coagulation factor VII and IX, lysozyme chloride, renin, bradykinin, tyrocidin, gramicidines, growth hormones, melanocyte stimulating hormone, thyroid hormone releasing hormone, thyroid stimulating hormone, parathyroid hormone, pancreozymin, cholecystokinin, human placental lactogen, human chorionic gonadotrophin, protein synthesis stimulating peptide, gastric inhibitory peptide, vasoactive intestinal peptide, platelet derived growth factor, growth hormone releasing factor, bone morphogenic protein, and synthetic analogues and modifications and pharmacologically-active fragments thereof.

Preferred peptide components of the compositions of the invention are synthetic analogues of LHRH, and particular such analogues include, but are not limited to, buserelin ([D-Ser(Bu^t)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), deslorelin ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), fertirelin ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), goserelin ([D-Ser(Bu^t)^6, Azgly^{10}]-LHRH), histrelin ([D-His(Bzl)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), leuprorelin ([D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), lutrelin ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), nafarelin ([D-Nal^6]-LHRH), tryptorelin ([D-Trp^6]-LHRH), and pharmacologically active salts thereof.

Suitable pharmacologically inactive basic peptides, which may be used in the salts of the invention, are polyarginine, polylysine and poly(arginine-co-lysine), (c -)polymers of neutral amino acids, in D-, L-

chains having carboxylic acid functionality. Thus the ring opening polycondensation of the acid dimers is carried out in the presence of an appropriate polymer chain transfer agent or co-initiator which controls both the molecular weight and the structure of the resulting homo- or co-polyester. Suitable such transfer agents are water, hydroxycarboxylic acids, monocarboxylic acids, dicarboxylic acids and polycarboxylic acids.

For polyesters prepared by polycondensation or co-polycondensation, the polymerisation is carried out under conditions such that an excess of carboxylic acid functionality is used, that is, the ratio of [-COOH] to [-OH] is equal to or greater than 1. The structure and molecular weight of the polycondensate are determined by the nature of the alcohols used (whether mono-ols, diols or polyols, or a mixture), the nature of the acids used (whether mono-, di- or poly-carboxylic acids, or a mixture), and the amount of the excess of carboxylic acid used. Acids involved in the Krebs cycle are particularly useful.

Examples of suitable hydroxy acids or lactones, which may be used to manufacture homo- or co-polyesters useful in this invention, include β-propionolactone, β-butyrolactone, γ-butyrolactone and pivalolactone, and α-hydroxybutyric acid, α-hydroxyisobutyric acid, α-hydroxyvaleric acid, α-hydroxyisovaleric acid, α-hydroxycaproic acid, α-hydroxyisocaproic acid, α-hydroxy-β-methylvaleric acid, α-hydroxyheptanoic acid, α-hydroxydecanoic acid, α-hydroxymyristic acid and α-hydroxystearic acid. Preferred such homo- and co-polyesters are those derived from lactic acid in its D-, L or DL- form, and glycolic acid, or the corresponding dimers lactide and glycolide, and a preferred optional chain stopper is lactic acid.

Although a macromolecular, basic peptide drug can exist wholly or in part as a polymer-cation, and a polyester can exist wholly or in part as a polymer-anion, salt formation arising from acid-base interaction between such polymeric species, using conventional processes of mixing, or using organic solvents, is extremely difficult or even impossible. For example, melt mixing the two components is unsuitable, since it is well known that peptides do not normally melt, but rather decompose at

sulfoxide, dimethylformamide, dimethylacetamide and N-methylpyrrolidone, have different problems because they are relatively non-volatile, have high boiling points, and so are extremely difficult to remove, and also because of the unacceptable toxicity of some of these solvents. It has been possible to identify certain solvents for both components which are more volatile and which are toxicologically acceptable, but such solvents present other difficulties. For example, acetic acid is a solvent for both peptides and polyesters, but the use of a large amount of acid solvent predisposes the peptide to exist as the acetate salt (because of mass action effects), so that the removal of the acetic acid at room temperature (say 20-25°C), or by freeze drying, results in phase separation of the peptide and the polyester, so that the desired salt formation tends not to occur.

It is an object of the present invention, therefore, to provide a process for the manufacture of a salt, comprising a cation of a basic peptide and an anion of a carboxy-terminated polyester.

The preparation of the peptide-polyester salts of this invention can be carried out using homo- or co-polyesters containing carboxylic acid groups, and peptides wherein the basic residues occur as the free base or as salts of a weak acid, preferably a volatile acid, having an acid dissociation constant of less than 10^{-3} or a pK_a ($pK_a = -\log_{10}K_a$, where K_a is the acid dissociation constant) of greater than 3. A particularly preferred such basic peptide salt is a salt with acetic acid. However, because of the inherent incompatibility of the two macromolecular species, particular conditions have to be used in which these peptide-polyester salts can be generated.

One means of achieving this is to use a solvent which dissolves both the peptide and the polyester, to form a solution, from which the solvent can be removed directly, leaving either firstly the amphipathic salt, or secondly a mixture of polyester and peptide in a physical state which is predisposed to form the amphipathic salt when processed further.

An example of the first approach is to use solvents such as, but

instantaneous, rate, and preferably at a temperature which is bel w the glass transition temperature of the polyester and the peptide. In this case, the solvent may be neutral or acidic, and a preferred solvent is acetic acid.

Such extremely rapid removal of solvent from a solution which exhibits some degree of viscous flow or visco-elastic behaviour results in phase separation of the two incompatible macromolecular types occurring on an extremely small colloidal scale. That is, the resulting peptide-polyester mixture has an extremely high surface area and surface energy. As a consequence, when another different solvent for the polyester, which is normally a non-solvent for the peptide, is added to essentially solvent-free peptide-polyester mixtures of this type, the high surface energy is dissipated by salt formation, and the disappearance of the colloidal nature of the peptide in the polyester. Suitable solvents for this second approach have to be freeze dryable and include, but are not limited to, acetic acid, dioxan/water mixtures and tert-butanol/water mixtures, or have to be spray dryable.

Thus, according to a further feature of this invention, there is provided a process for the manufacture of a salt comprising a basic peptide and a carboxy-terminated polyester, which comprises dissolving the basic peptide, in free base form or in the form of a salt with a weak acid, for example acetic acid, and the carboxy-terminated polyester in a solvent in which both are soluble, and which is capable of being removed by freeze-drying, freezing the resulting solution at high speed, freeze-drying the resulting frozen mixture, dispersing the resulting mixture in a solvent for the polyester component, and allowing the mixture to dissolve as the peptide-polyester salt is formed.

More particularly, in this process the solution of the peptide and the polylactic acid, or a co-polymer of lactic and glycolic acids, in acetic acid is added to liquid nitrogen in a dropwise fashion. This results in a more or less instantaneous freezing of the acetic acid solution, and a m re or less instantaneous generation of an essentially solvent-free peptide-polyester mixture. Freeze-drying to remove the

and $G^2_{solution} = G_0 + RT\ln C = G^1_{solid} + {}^{3\pi\gamma}/_{\sigma r}$,

or $G^1_{solid} = G_0 + RT\ln C - {}^{3\pi\gamma}/_{\sigma r}$.

But from (i) above,

$$G^1_{solid} = G_0 + RT\ln C_s,$$

and therefore

$$G_0 + RT\ln C - {}^{3\pi\gamma}/_{\sigma r} = G_0 + RT\ln C_s,$$

or $$C = C_s.e^{3\pi\gamma/\sigma r}$$

so that, as r decreases, C (hypothetically) increases.

In the usual case, higher than normal solubility due to small particle size is metastable, and the particles grow in size, for example by dissolution and recrystallisation, so that the effect of high surface energy is negated. However, with small particle size peptide-polyester mixtures, salt formation can occur, and this offers an alternative means of reducing the surface energy of the colloidal particles by allowing the formation of a soluble amphipathic salt, which as a solution offers the lowest free energy condition.

According to a further feature of the invention, there is provided a process for the manufacture of a salt comprising a basic peptide and a carboxy-terminated polyester, which comprises reacting a basic peptide in the form of a salt with a strong acid, such as a chloride or sulfate, with a polyester wherein some or all of the polyester is in the form of a carboxylic acid salt with a suitable alkali metal or alkaline earth metal, for example a sodium, potassium, calcium or magnesium carboxylate salt. For low molecular weight polyesters, (having a weight average molecular weight of less than about 10,000), the salts with alkalis can be dissolved, or very finely dispersed, in water. Addition of such a solution or dispersion to an aqueous solution (preferably free of carbon

However, even though transport of a peptide drug through a polyester by Fickian diffusion is essentially impossible for peptides of more than about 500Da or so, continuous release of polypeptides has nevertheless been achieved. European Patent No. 58,481 discloses how continuous release of a peptide drug from a polyester was obtained by using the very different properties of the two macromolecules, peptides being hydrophilic and water-soluble, and polyesters being hydrophobic and water-insoluble. In the formulations described in that patent, peptide drug release was achieved primarily through aqueous pores, which are generated initially by simple leaching of peptide from domains at the surface of the formulation, or from domains of peptide drug which are continuous or contiguous with the surface of the formulation. This leaching provides for an initial phase of release, and subsequent bulk hydrolytic degradation of the polyester results in the generation of further porosity within the polyester, and so further peptide release, governed by degradation and erosion, can occur. If the porosity arising from hydrolytic polyester degradation does not occur quickly enough, the initial release from the leaching phase is complete before sufficient degradation-induced porosity is generated in the delivery system, and discontinuous release of the peptide is obtained. The parameters of the formulations disclosed in EP 58,481 were therefore chosen so that hydrolytic degradation of the polyester occurred at the right time in relation to the initial leaching release phase, so as to ensure that the two phases of release overlapped, resulting in continuous release of the peptide drug.

However, whereas Fickian diffusional transport of a peptide through the polyester phase is impossible in the case of those simple peptide-polyester mixtures, a totally different situation arises in the case of formulations of the peptide-polyester salts of the present invention, optionally in the presence of free polymer. In formulations containing these materials, there is no separate phase consisting of polyester alone; rather, the continuous phase which controls release of the peptide is wholly or in part the peptide-polyester salt. Free peptide has some solubility in this phase of peptide-p lyester salt, and

administered by other routes. Of particular importance is the oral route, in which the various combinations of peptide-polyester salt and/or free peptide drug and/or free polyester can be used to good effect. In many instances, for oral administration it is preferred to use a pharmaceutically acceptable carrier such as a vegetable oil or a variant thereof, and including mono-, di- and tri-glycerides either alone or in admixture with other oils. Of less importance are the topical, rectal and intranasal routes of administration.

Other than European Patent No. 58,481 (1982), referred to above, Lavter et al. (loc. cit.) and Okada et al. (loc. cit.) are the only state of the art known to the applicants herein which refers to the possibility of obtaining peptide-polyester salts, but both these publications are speculative, in that they do not disclose how this putative interaction can be realised or utilized. It is a further object of the present invention to provide extended release pharmaceutical formulations, comprising various combinations of peptide-polyester salt and/or free peptide drug and/or free polyester in various proportions to give at least three different profiles of controlled drug release.

Thus, according to a further feature of the invention there is provided an extended release pharmaceutical composition comprising a peptide-polyester salt, as defined above, and/or free peptide drug and/or free polyester, and optionally other pharmaceutical excipient or excipients.

The design of the pharmaceutical compositions of this invention is based upon the following considerations. Whereas a simple peptide drug is normally soluble in water, both its salt with a polyester, and the free polyester itself, are normally totally water-insoluble, (although it is recognised that, for very low oligomeric forms of polyesters and co-polyesters, whilst they may themselves be water-insoluble, they may be water-soluble when in the form of a peptide-polyester salt). However, incubation of a mixture of a peptide drug and a polyester, wherein all or part of the peptide is present as the peptide-polyester salt, in aqueous physiological fluids, results in some degradation of the polyester. If

a wide or broad distribution or high polydispersity.

For the administration of peptide drugs by the parenteral route, such as intramuscular or sub-cutaneous injection or sub-dermal implantation of a depot or delivery system, polyesters having a number average molecular weight of more than 2000Da, or an inherent viscosity at 1%w/v at 25°C in chloroform of more than or equal to 0.08 dl/g, and up to and including 4.0dl/g, are preferred. For administration by other routes, such as orally, the preferred range of number average molecular weight is 500 to 5000Da.

It is obvious from the above considerations, which have largely been ignored in the state of the art, that the degradation of the polyesters, particularly in the presence of basic peptide, to give even a small fraction of water-soluble derived fragments, and the time interval for this to occur, will be controlled by molecular weight and molecular weight distribution. Essentially immediate degradation to water-soluble fragments occurs using both narrow and normal distribution polyesters, having weight average molecular weights of less than about 10,000Da and less than about 15,000Da respectively (depending on the type of molecular weight distribution), but in general the lower the polydispersity of the polyester the lower the weight average molecular weight required for immediate degradation to water-soluble fragments. For polyesters of weight average molecular weight of greater than 15,000Da, normal or wide distributions are required. Again this depends in part on the nature and type of the molecular weight distribution, but in general the higher the weight average molecular weight, the higher the polydispersity needed in order to achieve early degradation to water-soluble fragments.

For polyester or co-polyester and peptide compositions where some or all the peptide is in the form of a peptide-polyester salt, optionally containing free polyester, three different release profiles can be obtained. The first of these is when degradation of the polyester occurs to give essentially immediate generation of acidic water-soluble or hydrophilic fragments, which results in immediate release of peptide according to the following mechanism:-

it is only after some considerable time that the polyester degrades to water-soluble fragments and gives rise to free and transp rtable drug. This results in an extended induction period, during which there is initially no peptide release, following which induction period, release commences. This second case is ideal for timed and pulsed release of soluble vaccines and peptides.

The third case is when a formulation, based on a peptide-polyester drug system which contains a peptide drug both in its free form and in the form of a polymer-drug salt, optionally also in the presence of free polyester, and in which the polyester has a weight average molecular weight of greater than about 15,000Da, (and preferably greater than about 30,000Da), and having a narrow, or most probable, molecular weight distribution, is placed in a physiological environment, such as is found at intramuscular and sub-cutaneous injection sites, discontinuous release can result. A first phase of release arises because of the presence of free peptide drug, and its ability to be transported through the more permeable peptide-polyester salt system. If this first phase of release of free peptide drug is complete before degradation of the polyester in the peptide-polyester salt occurs to give further free peptide drug, then discontinuous peptide drug release will ensue.

Obviously, if there is no interval in which free peptide drug is absent from the composition, during its degradation, then continuous release will be obtained. This release profile is similar to that disclosed in European Patent No. 58,481, but the mechanism of release in European Patent No. 58,481 and the materials used (no peptide-polyester salt) are quite different from the mechanisms and materials defined in this application. Depending on release profile these mixtures are ideal for continuous release of peptides, proteins and soluble vaccines.

As stated above, these peptide-polyester drug salt systems, their physicochemical characteristics and the mechanisms by which release of the peptide occurs, are quite different from those disclosed in European Patents Nos. 58,481 and 52,510, and all other publications relating to peptide release from homo- and co-polymers of lactic and glycolic acids,

manufacture of pharmaceutical delivery systems. One of the most useful of these properties is the good solubility of the peptide, when in the form of a polyester salt, in organic solvents in which peptides are normally totally insoluble. This offers a great many advantages in pharmaceutical manufacture, in that it allows new processes and procedures to be used for the manufacture of drug delivery systems, and particularly facilitates aseptic manufacture. These processes and procedures, and the materials used, are totally different from the procedures and materials disclosed in the prior art.

Thus, solutions of a peptide-polyester salt, optionally containing free polymer, and/or free peptide in a solubilised or dispersed form, can be sterile-filtered, thus easing the problems normally associated with the sterile manufacture of solid or suspension peptide formulations. A sterile-filtered solution of a peptide-polyester salt can therefore be subjected to a variety of pharmaceutical drying procedures in an aseptic environment. Spray-drying, spray-congealing and other drying procedures which generate solid particles are preferred processes which readily lend themselves to aseptic operation.

Particularly useful is the generation of microparticles having particle sizes in the range from 0.2μm to 500μm, which can be suspended in a pharmaceutically acceptable injection vehicle. Such microparticles can be suspended in an aqueous injection vehicle prior to use, or alternatively in an organic injection vehicle which is a non-solvent for the materials used. For delivery systems based on homo- and co-polymers of lactic and glycolic acids, suitable such organic vehicles are highly lipophilic oils, such as (but not limited to) ethyl oleate, isopropyl myristate, vegetable oils and various fatty glycerides. In certain circumstances, it is preferred to use mixtures of such lipophilic vehicles.

Although such lipophilic vehicles are non-solvents for delivery forms based on lactic and glycolic acids, they are unsuitable for use with highly lipophilic polyesters such as those based on long chain hydroxy acids, for example hydroxystearic acids. For such highly

out effectively in the absence of carbon dioxide and in an inert atmosphere. It is further preferred that the organic solution f the peptide-polyester salt be free of carbon dioxide, because the concentration of carbon dioxide in air and water under normal conditions is sufficiently high, in comparison with the concentrations of polyester carboxylic acid groups, to enter into competitive salt formation due to mass action effects, according to the equation:-

$$P^-.D^+ + HCO_3^- \quad \xleftrightarrow{\text{------}} \quad D^+.HCO_3^- + P$$

where P is polyester and D is peptide drug. The resultant aqueous dispersions may then be dried by a variety of techniques, such as removal of the organic solvent in vacuo followed by freeze drying, or by directly removing both the solvent and the water in a single freeze drying operation. The resultant product may then be used to make suitable pharmaceutical preparations for injection in the manner described above.

A further alternative means of making microparticulate pharmaceutical formulations uses an essentially dry solution of the peptide-polyester salt, containing colloidally dispersed free peptide, in a suitable organic solvent or vehicle. (The term "essentially dry" is used, as it is virtually impossible to remove all traces of water from the peptide, and furthermore it means that none of the drug exists as an aqueous solution in a separate aqueous phase.) Addition of a non-solvent for the polymer, under conditions of vigorous agitation, followed by the addition of the solvent-swollen peptide-polyester salt (optionally containing free polymer and optionally containing free drug) to a large volume of a second non-solvent, to further harden and stabilise the precipitated microparticles, gives the final form. Obviously, under the appropriate conditions, or in the presence of a suitable surface active agents, such as (but not limited to) the fatty acid esters of sorbitol, the precipitation of the microparticles can be carried out using a single non-solvent for the polyester, for example a paraffin such as hexane.

The microparticles made by the various processes described herein are totally different structurally from the microcapsules prepared

as a gelled aqueous solution f drug, and the aqueous gelled phase was dispersed in a polymer solution. This water(aqueous drug gel)-in-oil (polymer solution) dispersion was then itself dispersed under shear in water, to give a water-in-oil-in-water double dispersion. After removal of the organic solvent under vacuum, and lyophilisation, microcapsules were obtained wherein the drug/gelling agent was encapsulated by polymer alone. The products of this process retain the drug as the simple salt, and not as the polymer salt of the peptide. The pharmaceutical formulations of the present invention therefore have structures, physicochemical characteristics and thermodynamic properties, which are totally different from the products described in European Patents Nos. 52,510, 145,240 and 190,833, wherein the microcapsules have the shape and geometry of microspheres in which a core, or cores, of drug is totally enclosed by polymer alone.

The products of this present application can also have the geometry and shape of (but are not limited to) microspheres, but either they are not microcapsules at all as defined above but rather are solutions of peptide-polyester salt (optionally also containing free polymer), or they are microcapsules wherein free peptide drug is encapsulated within a continuous phase or coating of the polymer-drug salt, optionally also containing free polymer. As indicated above, the permeability properties of such a polymer-drug salt are totally different from those of free polymer alone, so the products of the present invention release their peptide drug load in a manner which is totally different from those described in prior European Patents Nos. 52,510, 145,240 and 190,833.

Thus, a further embodiment of the invention is the preparation of either microspheres which are not microcapsules, using a solution of the peptide-polyester salt, optionally containing free polymer, or the preparation of microspheres which are microcapsules, but which comprise free drug encapsulated by a phase or coating of peptide-polyester salt, optionally containing free polymer.

Such diverse particles can be made by a variety of different

esters of citric acid, and low molecular weight (<1000) polyethylene glycols, alkoxypolyethylene glycols and polyethylene glycol acetates, etc., and of these benzyl benzoate and benzyl alcohol are preferred, especially benzyl benzoate.

The only requirement for such an organic solvent is that it is pharmaceutically acceptable and that the polyester-peptide drug salt is soluble in it. Whether or not a single such solvent is used, or a mixture of such solvents, the suitability of such solvents can be determined readily by simple experimentation. Homo- and co-polymers of lactic and glycolic acid are amongst the most polar and lipophobic polyesters, and so will not dissolve in such organic injection solvents as ethyl oleate, vegetable oils and other lipophilic carriers, but homo- and co-polymers based on lipophilic monomers or co-monomers, or lipophilic hydroxy acids such as hydroxystearic acid, are soluble in such lipophilic injection vehicles.

The ratio of peptide drug to polyester in the solids which are dissolved to form the solution composition of the invention, will naturally vary according to the potency of the peptide drug, the nature of the polyester used, and the period of peptide drug release desired.

The preferred level of peptide drug incorporation is from 0.1 to 30%w/v. In general, the optimal drug loading is dependent upon the molecular weight of the polyester and its molecular weight distribution, the period of release desired, and the potency of the peptide drug. Obviously, for drugs of relatively low potency, higher levels of incorporation may be required.

Water uptake by the composition is an important factor in controlling the rate of hydrolytic scission of the polyester, and the rate of water uptake is to some degree determined by the drug loading on the composition. Thus, in cases where relatively rapid drug release is required over a relatively short period, say three months, up to 30% peptide drug loading may be appropriate.

to administer by injection because of their viscosity. Thus solutions of ≤40% w/v are preferred for these polyesters. For solution compositions comprising polyesters of weight average molecular weight from about 8000 to about 20000, concentrations of ≤30 w/v are preferred, and for solution compositions comprising polyesters of molecular weight from about 20000 to about 50000, concentrations of ≤20% w/v are preferred. In some circumstances, for example if it is desired to inject the composition using a very narrow needle, very low viscosity solutions may be preferred, and the concentration could be reduced to 2% w/v or even less, but there will be a balance, of course, between reducing the viscosity and increasing the volume required to be injected.

According to a further feature of the invention, there is provided a process for the manufacture of a composition of the invention, which comprises:

1. dissolving an intimate mixture of the basic peptide drug and the polyester in the pharmaceutically acceptable solvent; or
2. slowly adding a solution of the peptide drug in a 1-6C alkanol to a solution of the polyester in a solvent suitable for injection, whereafter if the hydroxylic solvent is not pharmaceutically acceptable for injection it is removed by evaporation, or if the hydroxylic solvent is pharmaceutically acceptable for injection, its removal may not be necessary.

The intimate mixture of the basic peptide drug and the polyester, used in process 1. above, is preferably obtained by dissolving the basic peptide and the polyester in a solvent or solvent mixture which is capable of dissolving both the basic peptide drug and the polyester, and which is capable of being freeze-dried. Suitable examples of such solvents or solvent mixtures are glacial acetic acid and mixtures of dioxan and water, followed by freeze drying of the solution so obtained. Alternatively, the two components may be dissolved in for example dimethylsulfoxide, and the solvent subsequently removed.

The intimate mixture may also be obtained by dissolving the peptide drug in a hydroxylic solvent, for example methanol, and adding

mixtures of different types f implant. These can be prepared from the polyester-peptide drug salts of the invention, optionally containing free drug and/or free polyester, using conventional polymer melt-processing techniques, such as, but not limited to, extrusion, and compression and injection moulding, wherein elevated temperatures (preferably less than 100°C) are used to melt the polyester-drug salt in the preparation of the implant. Preparations of such implants can be carried out under aseptic conditions, or alternatively by terminal sterilisation by irradiation, using but not limited to γ- or X-rays. These solid dosage forms can be reduced to microparticulate forms by comminution or milling. The preferred particle sizes may range from 1μm to 500μm, and these microparticle delivery systems (which are neither microspheres nor microcapsules) can be suspended in a suitable conventional pharmaceutically acceptable injection vehicle.

The melt-processing of the peptide-polyester drug salt embodies and illustrates a most significant and important difference between the physicochemical and thermodynamic properties of the peptide-polyester drug salts of this invention, and the free peptides and simple salts thereof. The peptide-polyester salts of this invention in many instances melt and flow, in contrast to the free peptides and their simple salts, such as chlorides and acetates, which do not melt, but decompose at elevated temperature.

Degradation of polyesters is in part dependent on their molecular weight and polydispersity. Obviously, for degradation to occur mainly by hydrolytic scission of ester groups, the polyester or a pharmaceutical formulation containing a polyester, must take up water. For those systems where the release controlling matrix or membrane contains, in whole or in part, peptide-polyester drug salt, there will be a higher water uptake by the controlling matrix or membrane when compared to the polyester alone. Consequently, continuous matrix phases or membranes containing polyester-drug salt degrade differently from those continuous matrix phases or membranes based on polyester alone. It will also be understood that the rate of diffusion of water or physiological fluids into such a release controlling polyester matrix or membrane will control

have shown that for polyesters of similar molecular weight and molecular weight distribution the following general relationship applies in most cases for polyesters which are soluble in chloroform at 25°C, namely benzene-insoluble polyesters degrade faster than polyesters which are swollen but not dissolved by benzene, and such benzene-swellable polyesters degrade faster than those polyesters which are freely soluble in benzene, when degradation experiments are carried out in aqueous physiological fluids, or in buffer at pH 7.4 at 37°C. Consequently, it is particularly useful to use polyesters which are insoluble in benzene to provide continuous release of peptides from parenteral formulations over a relatively short period of time, say from one week to two months.

Thus, for compositions which may contain from 0.1% w/v of peptide up to 75% w/v of peptide, the following holds with respect to polyester composition, and its relations to structure, viscosity and polydispersity.

For the manufacture of peptide-polyester drug salts which can be formulated in accordance with this invention to give continuous drug release over a period of a week to two months, the molar composition of such benzene-insoluble polyesters, which preferably have a normal to wide polydispersity, preferably ranges from 60% glycolic acid (or glycolide)/-40% lactic acid (or lactide) to about 25% glycolic acid (or glycolide)/-75% lactic acid (or lactide), and such polyesters preferably have an inherent viscosity at 1% w/v in chloroform at 25°C ranging from 0.08 to 4.0dl/g.

By suitable choice of the polyester parameters, including molecular weight and molecular weight distribution, it is also possible to achieve continuous release of polypeptides over a period of one week to two months from formulations according to this invention, using polylactic acid homopolymer or co-polyesters having a molar composition ranging from 35% glycolic acid (or glycolide)/65% lactic acid (or lactide) to 10% glycolic acid (or glycolide)/90% lactic acid (or lactide), which are soluble in benzene, have an inherent viscosity at 1% in chloroform at 25° of from 0.08 to 0.5dl/g, and have a narrow to wide

polydispersity.

Continuous release of peptides over a relatively longer period of time, say 2 to 6 months, from formulations according to this invention, may be achieved using polylactic acid homopolymer or co-polyesters having a molar composition ranging from 35% glycolic acid (or glycolide)/65% lactic acid (or lactide) to 0% glycolic acid (or glycolide)/100% lactic acid (or lactide), which are benzene-soluble, have an inherent viscosity at 1% w/v in chloroform at 25°C of from 0.08 to 0.8dl/g, and have a narrow to wide polydispersity.

Continuous release of peptides over a very long period of time, say up to 2 years, from formulations according to this invention, may be achieved using polylactic acid homopolymer or co-polyesters having a molar composition ranging from 25% glycolic acid (or glycolide)/75% lactic acid (or lactide) to 0% glycolic acid (or glycolide)/100% lactic acid (or lactide), which are benzene-soluble, have an inherent viscosity at 1% w/v in chloroform at 25°C of from 0.2 to 4.0dl/g, and a normal to high polydispersity.

Timed or pulsed release (with an induction period prior to release), or discontinuous release (where there is an initial phase of release followed by a period of no release or ineffective release, followed by a second phase of release), over a relatively short period of time, say up to 2 months, may be acheived with the formulation according to this invention, using benzene-insoluble polymers which have a narrow to most-probable molecular weight distribution, and an inherent viscosity at 1% w/v in chloroform at 25°C from 0.3 to 4.0dl/g.

Yet another feature of the present invention, which is novel and distinguishes this invention from all other previously described controlled release drug delivery system based on polyesters or co-polyesters, and which further controls the rate of release, is the level of incorporation of peptide as the polyester salt (optionally in the presence of free drug and/or free polymer). This further controlling feature differs entirely from those parameters which result in increased

its variation with polyester molecular weight and polydispersity, is as follows. For continuous release of a peptide over very l ng periods of time, say up to 2 years, low levels of drug incorporation, ranging from 1.0% to 20% w/w, are preferred, using polyesters which have a preferred weight average molecular weight of 20,000Da or more and polydispersities greater than 2.2 and preferably greater than 3.5. These parameters for very long term release also depend in part on other features within the drug formulation, such as composition with respect to co-monomer content, structure, solubility/insolubility in benzene, and geometry and dimensions of the dosage form. A polyester of weight average molecular weight of about 20000 has an inherent viscosity of about 0.2, dependent upon such factors as its structure, composition and polydispersity.

For continuous release over relatively long periods of time, say up to 6 months, preferred levels of peptide drug incorporation range from 0.5% to 35% w/w, using polyesters or co-polyesters having weight average molecular weights of preferably 10,000Da or more, and polydispersities greater than 1.8 and preferably greater than 2.2, depending on all other parameters such as composition, structure, solubility/insolubility in benzene, and geometry and dimensions of the dosage forms.

For continuous release over relatively short periods of time, say up to 2 months, preferred levels of peptide drug incorporation range from 0.1% to 75% w/w, using polyesters having preferred weight average molecular weights of 2,000Da or more, and polydispersities greater than 1.2, depending on all other parameters such as composition, structure, solubility/insolubility in benzene, and geometry and dimensions of the dosage forms.

An additional parameter which further controls peptide drug release from formulations according to this invention, and which is absent from prior art types of delivery systems based on homo- and co-polymers of lactic acid and glycolic acid, is the functionality of the peptide, with regard to the number of basic groups such as arginine and lysine residues in the peptide drug molecule, and the functionality of the polyester or co-polyester with respect to the average number of

In the first of these instances (a pharmacologically active neutral polypeptide containing neither acidic nor basic residues) a salt of a synthetic polypeptide, which contains basic functionality and which is pharmacologically inactive, and the polyester, is used. Such a salt of the pharmacologically inactive synthetic polypeptide and the polyester or co-polyester is also amphipathic, and so can act as a dispersing agent for solubilising or colloidally dispersing a pharmacologically active, but neutral, peptide in an organic phase.

In the second of these cases, (where the pharmacologically active polypeptide contains residual carboxylic acid functionality), a salt of a synthetic polypeptide having at least two basic groups in the synthetic polypeptide chain, and which is pharmacologically inactive, and a polyester or co-polyester, is used. In this second case, in the salt of the synthetic polypeptide and polyester, the concentration of basic functional groups in the salt is greater than the concentration of carboxylic acid groups in the acidic, pharmacologically active peptide. This excess basic functionality in the salt can then interact by further salt formation with the carboxylic acid groups of the acidic pharmacologically active peptide. The resulting salts complex may then be solubilised or dispersed in an organic solvent or phase which is normally a total non-solvent for the peptide in question, but which are solvents for the polyester or co-polyester, in the manner described above for other polyester-peptide salts.

Because salts of peptides containing basic functionality with polyesters and co-polyesters containing carboxylic acid functionality are amphipathic, their surface active properties can be used to facilitate the dispersion of other hydrophilic drugs, or aqueous suspensions of such drugs, in an organic solvent or phase containing the polyester-peptide salt. The use of such amphipathic salts of peptides with polyesters or co-polyesters as dispersing or solubilising agents forms a further feature of this invention.

The invention is illustrated, but not limited, by the following

$$\frac{w \times 1000 \times f}{v \times n}$$

where w is the weight of polymer used,

f is the average number of carboxylic acid groups per polymer chain

v is the volume of sodium hydroxide used,

n is the normality of the sodium hydroxide used.

Example 1.

Goserelin acetate (100.6mg, equivalent to about 86mg of peptide as free base), and 50/50% molar D,L-lactide/glycolide co-polymer (300.3mg) containing one terminal carboxylic acid group per polymer chain and having a weight average molecular weight of 4300Da and an inherent viscosity at 1% w/v in chloroform at 25°C of 0.08dl/g, and which was insoluble in benzene, were dissolved in anhydride-free glacial acetic acid (3ml). The acetic acid solution of drug and polymer was added dropwise to liquid nitrogen, and the frozen droplets were freeze-dried for 24 hours under high vacuum conditions. The freeze-dried product was finally post-dried at 50°C for 24 hours under high vacuum, to give a polyester-drug mixture containing nominally about 25% w/w goserelin acetate (equivalent to about 22.3% w/w peptide as free base).

The dried polyester-drug mixture (400mg) was added to dichloromethane, and made up to 4ml. Initially, a cloudy colloidal mixture was obtained, but over the course of 1 hour this gradually cleared to form a clear solution. This solution was cast as a film, and allowed to dry at room temperature for about 6 hours, then for 20 hours at 50°C under high vacuum. A clear, transparent film containing polyester-drug salt was thus obtained.

(i) The clear, transparent film (100mg) thus obtained was melted and compression moulded at 80°C to give a transparent film, about 0.02cm thick. On immersion in water at 37°C for 24 hours, the weight of the hydrated drug/polymer film increased to 225mg. In contrast, the

0.02cm thick. The film was incubated in phosphate buffered saline (containing 0.02% sodium azide) at pH 7.4 and 37°C, and the buffer solution was assayed periodically by UV to determine the amount of goserelin released. This moulded product released goserelin continuously over about 2 weeks, and by 3 weeks had virtually degraded completely, and disappeared from the incubation medium.

This experiment demonstrates the utility of very low molecular weight, benzene-insoluble polymers for delivery of drug over a short time interval.

Similar moulded formulations can be manufactured using, in place of goserelin acetate, either naturally occuring gonadotrophin releasing hormones or other highly potent synthetic analogues (agonistic or antagonistic) of gonadotrophin releasing hormone, such as tryptorelin, leuprorelin, buserelin and nafarelin, preferably as the acetate salts or salts with other weak acids; or any other polypeptide hormone which controls secretion of the intact gonadotrophin or either of the gonadotrophin subunits.

Example 2

The clear, transparent film product obtained in Example 1 above (100mg) and a 50/50 molar D,L-lactide/glycolide co-polymer (1.05g) having a weight average molecular weight of 121,000Da and an inherent viscosity at 1% w/v in chloroform at 25°C of 0.84dl/g, and which is insoluble in benzene, were dissolved in dichloromethane (100ml). The solution was stirred vigorously at 1000 revolutions per minute (rpm), and silicone oil (50ml) was added slowly over 1 hour, to precipitate both the polyester-drug salt and the free polyester. After 1 hour, the partially precipitated mixture of polyester-drug salt, free polyester, silicone oil and dichloromethane was added to vigorously stirred hexane (2 litres) to harden the microparticles of polyester-drug salt and free polyester. This mixture was stirred for 2 hours and then allowed to settle, and the hexane layer was discarded. The microparticles (containing about 1.95% w/w goserelin as free base) were washed three times with fresh hexane

soluble in benzene, had a weight average molecular weight of about 5400Da, an inherent viscosity at 1% w/v in chloroform at 25°C of 0.08dl/g, and a polydispersity of 1.8, were dissolved in anhydride-free glacial acetic acid (4ml). This acetic acid solution of goserelin and polyester was added dropwise to liquid nitrogen, and the frozen droplets were isolated, freeze-dried under vacuum for 24 hours, and then dried at 55°C for 24 hours under high vacuum.

(i) The resulting dried product was added to dichloromethane (4ml), to give a cloudy mixture initially, which rapidly dissolved to give a clear solution which was filtered through a 0.2µm nylon sterilising filter.

This experiment shows that solutions of the polyester salt of goserelin can be sterile-filtered, in contrast to mixtures or dispersions of simple drug salts in an organic solution of the polyester.

(ii) Trifluoroacetic acid (50µl) was added to the clear dichloromethane solution from (i) above (1ml), with vigorous agitation. There was an immediate precipitation of goserelin as its trifluoroacetate salt, showing that the goserelin was present in the dichloromethane solution as the salt with the carboxy-terminated polyester.

Similar sterile solution formulations can be manufactured using, in place of goserelin acetate, either naturally occurring gonadotrophin releasing hormones or other highly potent synthetic analogues (agonistic or antagonistic) of gonadotrophin releasing hormone, such as tryptorelin, leuprorelin, buserelin or nafarelin, preferably as the acetate salts or salts with other weak acids; or any other polypeptide hormone which controls or modulates secretion of the intact gonadotrophins or either of the individual gonadotrophin sub-units.

Example 4.

The dichloromethane solution of goserelin-polyester obtained in Example 3 (2ml) was diluted with more dichloromethane and made up to 10ml. This solution was sprayed into vigorously stirred hexane (1

Example 5.

Goserelin acetate (304mg, equivalent to about 248mg of goserelin as free base) and 100% molar poly(D,L-lactic acid) (102mg), having a weight average molecular weight of about 5400, an inherent viscosity at 1% w/v in chloroform at 25°C of 0.08dl/g, and a polydispersity of 1.8, were dissolved in anhydride-free glacial acetic acid (2ml). The acetic acid solution of goserelin and polyester was then added dropwise to liquid nitrogen, and the frozen droplets were isolated, freeze-dried under high vacuum for 24 hours, and then dried under vacuum at 55°C for 24 hours.

The resulting product was added to dichloromethane (2ml) to give a cloudy, colloidal mixture which did not clear totally with time. This mixture in dichloromethane comprised essentially a dispersion of goserelin acetate in the goserelin-polyester salt.

This dispersion of goserelin acetate in the methylene chloride solution of the polyester-goserelin salt was formulated into a microparticulate form, containing goserelin equivalent to about 72% w/w as free base, wherein the free goserelin acetate is dispersed throughout a continuous phase of the goserelin-polyester salt, by spray drying, spray-congealing, simple precipitation or by phase separation co-acervation.

Similar microparticle formulations may be manufactured by using, in place of goserelin acetate, either naturally occuring gonadotrophin releasing hormones or other highly potent synthetic analogues (agonists or antagonists) of gonadotrophin releasing hormones, such as tryptorelin, leuprorelin, buserelin or nafarelin, preferably as the acetate salts or salts with other weak acids; or any other polypeptide hormone which controls or modulates secretion of the intact gonadotrophins or either of its individual sub-units.

The dried goserelin-polyester mixture, prepared as described above, (1g) was dissolved in 8ml of dichloromethane. The resulting solution was placed in a 250ml multinecked round-bottomed flask and swept with a stream of nitrogen to remove all air, and to generate a carbon dioxide-free atmosphere. Water (90ml), which had previously been degassed to remove all carbon dioxide and then stored under carbon dioxide-free nitrogen, was introduced into the flask, and the mixture was stirred vigorously at about 500 rpm under an atmosphere which was essentially carbon dioxide-free. The dichloromethane solution of goserelin-polyester salt rapidly dispersed to give a stable oil (dichloromethane solution of drug-polymer salt)-in-water dispersion. Whilst maintaining stirring at about 200 rpm, a vacuum was gradually applied and the bulk of the dichloromethane was evaporated under vacuum, to give a dispersion of goserelin-polyester salt in water. Freeze-drying this dispersion produced microparticles, in which the goserelin is present as the goserelin-polyester salt having an average particle size of about 20μm, which was shown to release goserelin over about 6 weeks, when incubated in saline, buffered with phosphate to pH 7.4 at 37°C, and the supernatant periodically assayed by UV for goserelin.

Similar microparticles may also be manufactured by incorporating in the aqueous phase agents which are known to improve polypeptide stability such as mannitol. Although it is preferred to carry out the above process in a carbon dioxide-free atmosphere, it is nevertheless possible to achieve satisfactory results in the presence of traces of carbon dioxide, depending on polyester molecular weight and drug loading.

Similar sterile solution, cast film and microparticle formulations may be manufactured in a similar manner using, in place of goserelin acetate, either the natural analogues of gonadotrophin releasing hormones or other highly potent synthetic analogues (agonists or antagonists) such as tryptorelin, leuprorelin, buserelin or nafarelin, preferably as acetate salts or salts with other weak acids; or any other polypeptide hormone which can control or modulate the secretion of intact gonadotrophins or either of their sub-units.

hormone, such as tryptorelin, leuprorelin, buserelin or naferelin, preferably as the acetate salts or salts with other weak acids; or any other polypeptide hormone which can control or modulate the secretion of intact gonadotrophins or either of their sub-units.

Example 8.

Goserelin acetate (771mg, equivalent to about 670mg of goserelin as free base), 95/5 molar D,L-lactide/glycolide co-polymer (1.8g) having a weight average molecular weight of about 3600Da and an inherent viscosity at 1% w/v in chloroform at 25°C of 0.08dl/g, and 95/5 molar D,L-lactide/glycolide co-polymer having a weight average molecular weight of about 15,000Da and an inherent viscosity at 1% w/v in chloroform at 25°C of 0.17dl/g (4.2g), were dissolved in anhydride-free glacial acetic acid (70ml). The combined polymers had a weight average molecular weight of about 12,300Da and a polydispersity of about 2.6. The goserelin-polyester solution was added dropwise to liquid nitrogen, and the frozen droplets were isolated and freeze-dried under high vacuum for about 18 hours. The product drug-polymer mixture was finally dried at 55°C for 24 hours under high vacuum.

The dried drug-polymer mixture (6g) was added to dichloromethane (60ml) to give an initially cloudy colloidal mixture which, over the course of 1 hour, gradually cleared to give a clear solution of goserelin-polyester salt in dichloromethane.

This solution was spray-dried using a Buchi spray dryer, using an inlet temperature of 60°C and an outlet temperature of 35°C, to produce approximately spherical microparticles of about 1μm to about 10μm diameter.

In these microparticles the drug is present essentially completely as the goserelin-polyester salt, as the acetic acid content, as free acid or anion, is 0.06% or less, instead of 0.6 to 0.7% which would be required if the goserelin were present as its acetate salt.

amounts of drug are released.

(i) The microparticles obtained in Example 8 (450mg) were dispersed in water containing 2% w/v of sodium carboxymethyl cellulose and 0.2% w/v polysorbate 80, and made up to 3ml with water. 0.2ml (equivalent to about 3mg of goserelin as free base) was injected sub-cutaneously into 10 normal adult female rats showing regular cyclicity, and the ensuing effect on oestrous cyclicity was determined by microscopic examination of vaginal smears. The animals entered a continuous phase of dioestrous, that is chemical castration, lasting 95 ± 3 days.

This experiment shows that an aqueous suspension formulation of goserelin-polyester salt, based on a lov molecular weight benzene-soluble polyester, provides a relatively long period of controlled release of about three months of a peptide drug which has a metabolic half-life of only 4-6 hours.

(ii) The microparticles obtained in Example 8 (450mg) were dispersed in ethyl oleate, and made up to 3ml. Again 0.2ml of formulation were administered to (six) female rats showing regularly cyclicity by subcutaneous injection. The animals entered a continuous phase of dioestrous lasting 81 ± 3 days.

This experiment shows that a solution formulation of goserelin-polyester salt in an organic injection vehicle, which is a non-solvent for the polyester alone, provides a relatively long period of controlled peptide drug release.

Example 10.

Leuprorelin acetate (50.3mg) and the co-polyester comprising 78% molar D,L-lactic acid and 22% molar glycolic acid, described in Example 6 above (453.2mg), were dissolved in anhydride-free glacial acetic acid (5ml). The resulting solution was added dropwise to liquid nitrogen, and the frozen droplets were freeze-dried under high vacuum for 22 hours, and then further dried at 55°C for 24 hours under high vacuum.

controls secretion of the intact gonadotrophins or either of the gonadotrophin sub-units.

Example 11.

i) Goserelin acetate (2.28g, equivalent to about 2.00g of goserelin as free base) was dissolved in anhydride-free glacial acetic acid (60ml). A mixture of two 95/5% molar poly(D,L-lactic acid)/polyglycolic acid) copolymers (12.6g of a copolymer with a weight average molecular weight of 15,846 and a polydispersity of 1.38, and 5.4g of a copolymer with a weight average molecular weight of 3,896 and a polydispersity of 1.78) and therefore providing an excess of copolymer carboxylic acid end groups relative to basic drug, was dissolved with stirring in anhydride-free glacial acetic acid (150ml) to give a clear solution. The drug solution was added to the copolymer solution and was mixed thoroughly. This mixture was then added dropwise to liquid nitrogen to freeze it as small beads, and the solid material was freeze dried for two days using an Edwards high vacuum freeze drier. The dried material was further dried at 50-55°C in a vacuum oven for 24 hours.

This dried product (100mg) was added to dichloromethane (1ml) and was found to dissolve totally within 2 hours to give a clear solution. It is shown by this Example that the formation of the polyester-goserelin salt confers good solubility upon the drug such that it can be dissolved in a non-polar solvent.

ii) Goserelin acetate (2.28g, equivalent to about 2.00g of goserelin as free base) was dissolved in anhydride-free glacial acetic acid (60ml). A mixture of two 100% molar poly(D,L-lactic acid) polymers (12.6g of a polymer with a weight average molecular weight 15,178 and a polydispersity of 1.27, and 5.4g of a polymer with a weight average molecular weight of 4,204 and a polydispersity of 1.84) and therefore providing an excess of copolymer carboxylic acid end groups relative to basic drug, was dissolved with stirring in anhydride-free glacial acetic acid (150ml) to give a clear solution. The drug solution was added to the polymer solution and was mixed thoroughly, and this mixture was then added

copolymers (12.0g of a copolymer with a weight average molecular weight of 35,833 and a polydispersity of 1.83, and 5.15g of a polymer with a weight average molecular weight of 4,116 and a polydispersity of 1.86) and therefore providing an excess of polymer carboxylic acid end groups relative to basic drug, was dissolved with stirring in anhydride-free glacial acetic acid (150ml) to give a clear solution. The drug solution was added to the copolymer solution and was mixed thoroughly. This mixture was then added dropwise to liquid nitrogen to freeze it as small beads. The solid material was freeze dried for two days using an Edwards high vacuum freeze drier, and the dried material was further dried at 50-55°C in a vacuum oven for 24 hours.

This dried product (100mg) was added to dichloromethane (1ml) and was found to dissolve totally within 10 minutes to give a clear solution. It is shown by this Example that the formation of the polyester-goserelin salt confers good solubility upon the drug, such that it can be dissolved in a non-polar solvent.

Comparative Example

Goserelin acetate (2.28g, equivalent to about 2.00g of goserelin as free base) was dissolved in anhydride-free glacial acetic acid (60ml). A 50/50% molar poly(D,L-lactic acid)/polyglycolic acid) copolymer (18.0gm polymer with a weight average molecular weight 22,307 and a polydispersity of 2.07) and therefore providing an approximately stoichiometric equivalent of copolymer carboxylic acid end groups relative to basic drug, was dissolved with stirring in anhydride-free glacial acetic acid (150ml) to give a clear solution. The drug solution was added to the copolymer solution and was mixed thoroughly. This mixture was then added dropwise to liquid nitrogen to freeze it as small beads. The solid material was freeze dried for two days using an Edwards high vacuum freeze drier, and the dried material was further dried at 50-55°C in a vacuum oven for 24 hours.

This dried product (100mg) was added to dichloromethane (1ml) and

that this causes the solubility properties of the drug in non-p lar solvent to return to that expected of the acid salt of a peptide drug (i.e. not soluble).

Example 12.

The spray dried particles i-iv in Example 11 were dispersed (18% v/v) in an aqueous vehicle suitable for injection (2% sodium carboxymethylcellulose [Fluka, medium viscosity], 0.2% polysorbate 80 [Tween (trade mark), Fluka].

The spray dried particles from Example 11, dispersed in the injection vehicle described above, were injected into ten female Wistar-derived rats. Blood samples were taken from the tails of five rats on days 7, 14 and 28, and these samples were assayed for goserelin using a radioimmunoassay with known specificity for the drug and proven lack of cross reactivity to metabolites.

The results of these experiments showed that this formulation achieved measurable blood levels of goserelin for at least 4 weeks.

Example 13.

Spray dried product ii of Example 11 was dispersed in the following aqueous vehicles for injection.

a. sodium carboxymethyl cellulose (medium viscosity grade, Fluka) 1.0%, and polysorbate 80 (Tween) 0.75%.

b. methyl cellulose (15mPa.s, Fluka) 0.75% and polysorbate 80 (Tween) 0.75%.

These formulations dispersed well in these vehicles, and were suitable for parenteral administration.

then filtered (Millex 0.5µm) and the filtrate assayed for drug content by HPLC. The release profile of the depots was calculated by reference to the drug content of depots which had not been implanted, and which were included in the same assay. These depots of drug-polyester salt gave sustained release of goserelin in vivo for a period of at least four weeks.

Example 16

(i) Lactide/glycolide copolymer (95/5) with a single terminal carboxylic acid group (8.87g, Mw = 5750, polydispersity = 1.5, molecular weight by end group titration = 2516g/mole, inherent vicosity at 1% w/v in chloroform = 0.10 dl/g) was dissolved in dichloromethane (50ml) with stirring. To this was added 1.13g goserelin acetate, forming a cloudy suspension. Methanol (5ml) was added with stirring, and after 30 minutes the mixture was completely clear. The solvent was then removed from the solution by rotary evaporation to leave a clear solid. This solid was redissolved in dichloromethane (50ml) and the solvent was again removed by rotary evaporation. The redissolution step and solvent removal step were repeated twice more to leave a very viscous fluid which was dried under high vacuum to give a white foam. The foam was broken up and dried under vacuum for a further 24 hours at room temperature to give a fine amorphous solid.

(ii) The process described in i) above was repeated, using a lactide/glycolide copolymer (75/25) with a single terminal carboxylic acid (8.87g, Mw = 10900, polydispersity = 1.85, molecular weight by end group titration = 3210g/mole, inherent viscosity at 1% w/v in chloroform = 0.14dl/g), to give a fine amorphous solid.

Formulation 1

The goserelin-lactide/glycolide polymer salt from (i) above (1g) was added to benzyl benzoate (99%, ex Janssen, 2ml) and this was heated using a hand held hot-air gun whilst agitating the mixture until the solid was dissolved. 110µl of this solution formulation contained 3.6 mg

The normal oestrus cycle (oestrus, dioestrus, met-oestrus, pro-oestrus), can be followed from the proportions of the various cell types (leucocytic, epithelial and cornified) in the smear. If the release of drug from the formulations is continuous the normal oestrus cycle is interrupted and the rats will remain in dioestrus as long as release of the goserelin continues.

Formulations 1-6 were dosed to groups (n=6) of regularly cycling female rats at a dose of 3.6mg goserelin per rat. A syringe fitted with a 20 gauge needle was used for dosing the formulations subcutaneously. An undosed group of five rats was used as a control group. Vaginal smears were taken daily from the rats, and examined to determine the oestrus state of the animals, and the results obtained were as follows:

Formulation number	Average duration of dioestrus (days) (± s.e.)
1	100 ± 2.7
2	120 ± 6.3
3	69 ± 5.9
4	59 ± 1.2
5	61 ± 2.1
6	53 ± 3.7

From these results it can be seen that all six formulations gave periods of goserelin release in excess of 6 weeks and that formulations 1 and 2 released goserelin for a period of three months or more. It can further be seen from these examples that the formulations of the goserelin-polyester salt can be provided as solutions which can be readily administered parentally using a narrow gauge needle, and that such formulations are convenient for treatment of hormone dependent tumours in man.

Example 17

Formulation 1

in which goserelin acetate itself is not soluble.

Biological evaluation

Formulations 1-3 were dosed to groups (n=10) of regularly cycling female rats at a dose of 3.6mg goserelin per rat, as described in Example 16. Following dosing, the animals were found to enter a period of continuous dioestrus indicating continuous release of goserelin. The average duration of the diostrus period for each group of rats is given in the following table. From this table it can be seen that all three formulations gave periods of goserelin release in excess of fourteen weeks.

Formulation No.	Average duration of dioestrus (days) (± s.e.)
1	104 (± 5.4)
2	99 (± 3.9)
3	101 (± 2.8)

It can further be seen from these examples that the formulations of the goserelin polyester salt can be provided as solutions which can be readily administered parentally using a narrow gauge needle, and that such formulations are convenient for the treatment of hormone dependent tumours in man.

Example 18

Formulation 1

Lactide/glycolide copolymer (95/5) with a single terminal carboxylic acid (4.5g, Mw = 6806, polydispersity = 1.55, molecular weight by end group titration = 3027g/mole, inherent vicosity at 1% w/v in chloroform = 0.108dl/g) was dissolved in glacial acetic acid (50ml). To this solution was added goserelin acetate (0.56g, equivalent to 0.5g

This goserelin-lactide/glycolide copolymer mixture (0.36g) was added to benzyl benzoate (2.64ml, 99%, ex Janssen) and was dissolved with warming and agitation. The final solution contained 3.47mg of goserelin in 110µl.

Formulation 4

The process described above for Formulation 1 was repeated, using a lactide/glycolide copolymer (95/5) with a single terminal carboxylic acid (8.66g, Mw = 5604, polydispersity = 1.71, molecular weight by end group titration = 1960g/mole, inherent vicosity at 1% w/v in chloroform = 0.094dl/g and 1.08g of goserelin acetate (equivalent to 0.96g of goserelin). The acetic acid content of this freeze dried solid was determined by gas chromatography and was found to be 0.08% and the goserelin content of the final product was 9.90% w/w.

This goserelin-lactide/glycolide copolymer mixture (1.0g) was added to benzyl benzoate (2.0ml, 99%, ex Janssen) and was dissolved with warming and agitation. The final solution contained 3.67mg of goserelin in 110µl.

Biological evaluation

Formulations 1-4 were dosed to groups (n=9 or 10) of regularly cycling female rats at a dose of 3.6mg goserelin per rat, as described in Example 16. Following dosing, the animals were found to enter a period of continuous dioestrus indicating continuous release of goserelin. The average duration of the diostrus period for each group of rats is given in the following table. From this table it can be seen that all three formulations gave periods of goserelin release for a period of about 3 months or more.

Formulation 2

1g of the solid was added to benzyl alcohol (distilled, bp 44°C at 0.3mb, 1.7ml) and was warmed using a hot-air gun until dissolved. 100µl of this solution formulation contained 3.6 mg of goserelin.

Biological evaluation.

Two groups of ten female rats were dosed subcutaneously using a 20 gauge needle with formulations 1 and 2 at a dose of 3.6 mg per rat. Terminal blood samples were taken from the rats at subsequent timepoints (1 week (n=4), 4 weeks and 6 weeks (n=3)). The blood samples were assayed for goserelin by means of radioimmunoassay. Measurable blood levels of goserelin were found with both formulations, indicating that the solution formulations gave sustained drug release for several weeks. The blood level profile of formulation 1 was found to peak at about four weeks, whereas with formulation 2 the peak occurred at week one and thereafter the blood levels were found to decline progressively with time. The blood level profile of formulation 1 is considered to be more desirable than that of formulation 2 due to the more constant blood levels obtained when benzyl benzoate is used as the solvent for the solution formulation.

It can further be seen from these examples that the formulations of the drug polyester salt can be provided as solutions which can be readily administered parentally using a narrow gauge needle, and that such formulations are convenient for treatment of hormone dependent tumours in man.

Example 20

A lactide/glycolide copolymer (95/5) with a single terminal carboxylic acid (9.0g, M_w = 6011, polydispersity = 1.56, molecular weight by end group titration = 2700g/mole, inherent vicosity at 1% w/v in chloroform = 0.099dl/g) was dissolved in dichloromethane (100ml). To this was added goserelin acetate (1.124g, equivalent to 1g of goserelin)

and remained as a cloudy suspension.

A lactide/glycolide copolymer (70/30) with a single terminal carboxylic acid (225mg, Mw = 9755, polydispersity = 1.52, molecular weight by end group titration = 1800), was added to dichloromethane (25ml). This was stirred for 15 minutes to give a clear colourless solution. To this was added a solution of Substance P (25mg) in methanol (0.5ml). The resulting cloudy suspension was stirred for 1 hour, by which time a completely clear solution had formed. The solvent was removed by rotary evaporation and the clear 'glassy' solid obtained was redissolved in dichloromethane (5ml) and reevaporated. This was repeated twice. The final solid was dissolved in dichloromethane (3ml) and the solution was dropped slowly onto PTFE coated cloth, alloving the solvent to evaporate to form a thin film of a clear colourless glassy solid (peptide content 9.1% w/w).

This film (96.8mg) was placed in a small vial and phosphate buffered saline (2ml, pH 7.4) was added (buffer was previously filtered through a 0.2μm filter and contained 0.02% sodium azide as a preservative). The vial was placed in an incubator at 37°C and the buffer was removed and replaced periodically. The buffer which was removed was analysed for release of Substance P, using an ultraviolet spectrophotometer (Hewlett Packard 8452A) at 210nm, against standard solutions of substance P. The results show that Substance P can be dissolved in dichloromethane when formed as the salt of a carboxy-terminated lactide/glycolide copolymer, and can be processed in this solvent to give a thin film, which gives continuous release of the peptide for a period of about 4 weeks.

Example 22

An aqueous solution of leuprolide acetate (otherwise known as leuprorelin acetate), (300μl of a 330mg/ml solution) is added under high shear conditions, to 20ml of a 10% w/w solution of poly(hydroxystearic acid) having a number average molecular weight of about 2000, in Miglyol 812 (triglycerides of medium chain saturated fatty acids including

dichloromethane, when formed as the salt of a carboxy-terminated lactide/glycolide copolymer, and that the resulting mixture gives continuous release of the peptide for a period of at least four weeks.

Release of Lys^8-vasopressin in vitro

Time (days)	Release of Lys^8-vasopressin from film (%)
1	4.11
4	5.45
7	5.55
14	5.75
21	26.82
28	47.27

Example 24

Two formulations of ZENECA ZD6003 ([Met^{-1}, Arg^{11}, $Ser^{17,27,60,65}$] human G-CSF (granulocyte-colony stimulating factor) modified with polyethylene glycol 5000 as described in Reference Example 4 or 7 of European Patent Publication No. 0 473 268) in lactide/glycolide copolymer were prepared as follows.

(i) Dichloromethane (4ml) was added to a freeze-dried preparation of ZD6003 (39.72mg). This resulted in an opaque dispersion of drug in the solvent. A lactide/glycolide copolymer (75/25) with a single terminal carboxylic acid (363.6mg, Mw = 9963, polydispersity = 2.19, molecular weight by end group titration = 2815) was added, and a clear solution formed.

This solution was added to a s lution (400ml) of methyl cellulose (0.25% w/v Methocel, 15mPa.s, ex Fluka) in water under shear (2150 RPM, Heidolph RZR50 stirrer). After stirring at this rate for 3 minutes the stirring speed was reduced to 800RPM. The resulting particles were then

Particles made in this way were of inferior quality, compared with those obtained in (i) above, with some being of irregular shape and of a mean size of 40μm as determined by image analysis from optical microscopy. The drug content of these particles was determined by extraction followed by HPLC analysis and was found to be 2.05%, representing an incorporation efficiency of 19% of the drug used to form the microparticles.

The above example shows that ZD6003 can be dissolved in dichloromethane when in the presence of a polymer with a single terminal carboxylic acid, despite dichloromethane iself being a non-solvent for the drug. In addition such a solution can be used to form microparticles of drug and polymer with a very high rate of incorporation of drug. In contrast, the above example also shows that ZD6003 cannot be dissolved in dichloromethane in the presence of a polymer, when such a polymer does not have a terminal carboxylic acid, and forms only a hazy dispersion. Furthermore such hazy dispersions of ZD6003 in a solution of polymer with no terminal carboxylic acid result in poor incorporation of drug when processed to form microparticles.

Example 25

(i) Goserelin acetate (22.47mg, equivalent to 19.99mg goserelin) was added to benzyl benzoate (2.21g, 99%, ex Janssen). This mixture was placed in an incubator at 40°C and was stirred continuously for 9 days using a magnetic stirrer. After 2 and 9 days aliquots were taken and centrifuged for 15 minutes at 13,000 RPM to pellet undissolved drug. Aliquots of supernatant (approx. 100mg) were weighed accurately into 50ml volumetric flasks. To each was added glacial acetic acid (2ml), followed by making up to volume with an aqueous solution of trifluoroacetic acid (0.5% v/v). A portion of this solution was placed in a centrifuge tube and was centrifuged at 13,000 RPM for 15 minutes to separate suspended material. The supernatant was then assayed for goserelin content, using HPLC. N goserelin was detectable in either sample. The limit f detection of goserelin in this HPLC assay was 0.2ug/ml and the limit of quantification was 0.5μg/ml. Thus the equilibrium solubility (at 40°C)

supernatant was carefully removed and weighed into a 50ml volumetric flask. The sample was assayed for goserelin content as described in (i). The goserelin content of this solution was found to be 24.6µg/mg benzyl benzoate.

This example shows that benzyl benzoate is a very poor solvent for goserelin acetate. Furthermore, the addition of a lactide/glycolide polymer to form a simple mixture with goserelin acetate in benzyl benzoate does not lead to a marked increase in the equilibrium solubility of goserelin acetate in benzyl benzoate. However, goserelin/polyester salt could be dissolved in benzyl benzoate to form a solution containing goserelin at a concentration very much higher than the estimated equilibrium solubility of free goserelin in this solvent.

D-, L- or DL-form, with arginine and/or lysine in D-, L- or racemic form, or peptides or (co-)polypeptides in which the peptide chains are terminated in whole or in part by a basic group at the N-terminus and the backbone is comprised of neutral amino acid residues.

4. A composition as claimed in claim 1 wherein the polyester is selected from those derived from hydroxy-acids and those derived from the polycondensation of diols and/or polyols with dicarboxylic acids and/or polycarboxylic acids.

5. A process for the manufacture of a solution or dispersion of a salt as claimed in claim 1, which comprises

(a) dissolving the peptide containing at least one basic amino acid, in free base form or in the form of a salt with a weak acid and the carboxy-terminated polyester in a neutral, polar solvent in which both are soluble, removing the solvent or most of the solvent, and adding the remaining concentrated solution to an excess of a non-solvent for the peptide-polyester salt, or

(b) dissolving the peptide containing at least one basic amino acid, in free base form or in the form of a salt with a weak acid, and the carboxy-terminated polyester, in a solvent in which both are soluble, and which is capable of being removed by freeze-drying, freezing the resulting solution at high speed, freeze-drying the resulting frozen mixture, dispersing the resulting mixture in a solvent for the polyester component, and allowing the mixture to dissolve as the peptide-polyester salt is formed, or

(c) reacting the peptide, containing at least one basic amino acid, in the form of a salt with a strong acid, with a polyester wherein some or all of the polyester is in the form of a carboxylic acid salt with a suitable alkali metal or alkaline earth metal.

6. A composition as claimed in claim 1, comprising a pharmacologically active peptide and a polyester, for extended release of the peptide drug, characterised in that the composition is in the form of microparticles from 0.2μm to 500μm in diameter, suspended in a pharmaceutically acceptable injection vehicle.

11. A composition as claimed in claim 8 wherein the ratio of basic peptide drug-polyester salt to free polyester is from 1:0 to 0.1:10.

12. A composition as claimed in claim 8 wherein the ratio of total solids to solvent is from 2% w/v to 40% w/v.

13. A process for the manufacture of a pharmaceutical composition as claimed in claim 8 which comprises

(a) dissolving an intimate mixture of the peptide drug and the polyester in the pharmaceutically acceptable solvent; or

(b) slowly adding a solution of the peptide drug in a 1-6C alkanol to a solution of the polyester in a solvent suitable for injection, whereafter, if the solvent in the starting peptide solution is not pharmaceutically acceptable for injection, it is removed.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	PHARMACEUTICAL RESEARCH vol. 8, no. 5, 1991, pages 584 - 587 H. OKADA ET AL. 'SUSTAINED PHARMACOLOGICAL ACTIVITIES IN RATS FOLLOWING SINGLE AND REPEATED ADMINISTRATION OF ONCE-A-MONTH INJECTABLE MICROSPHERES OF LEUPROLIDE ACETATE' cited in the application see the whole document	1-', 6-7
Y	EP,A,0 058 481 (IMPERIAL CHEMICAL INDUSTRIES PLC) 25 August 1982 cited in the application see abstract see page 4, line 17 - page 5, line 18 see page 7, line 5 - line 12 see page 18, line 20 - line 23; claims	1-13
Y	US,A,4 997 643 (PARTAIN ET AL.) 5 March 1991 see abstract see column 6, line 33 - column 7, line 62; claims	1-13
Y	EP,A,0 467 389 (UNIVERSITY OF KENTUCKY RESEARCH FOUNDATION) 22 January 1992 see abstract see page 4, line 53 - page 5, line 6 see page 5, line 24 - line 44 see page 6, line 19 - line 56 see page 8, line 14 - line 27; claims; examples 1-2,5	1-13
A	EP,A,0 052 510 (SYNTEX INC.) 26 May 1982 cited in the application see abstract; claims	1-13
A	ZIEKENHUISFARMACIE vol. 4, no. 2, 1988, pages 54 - 56 F.G. HUTCHINSON ET AL. 'BIODEGRADABLE POLYMERS FOR THE DELIVERY OF POLYPEPTIDES AND PROTEINS' see the whole document	1-13